FIG. 1

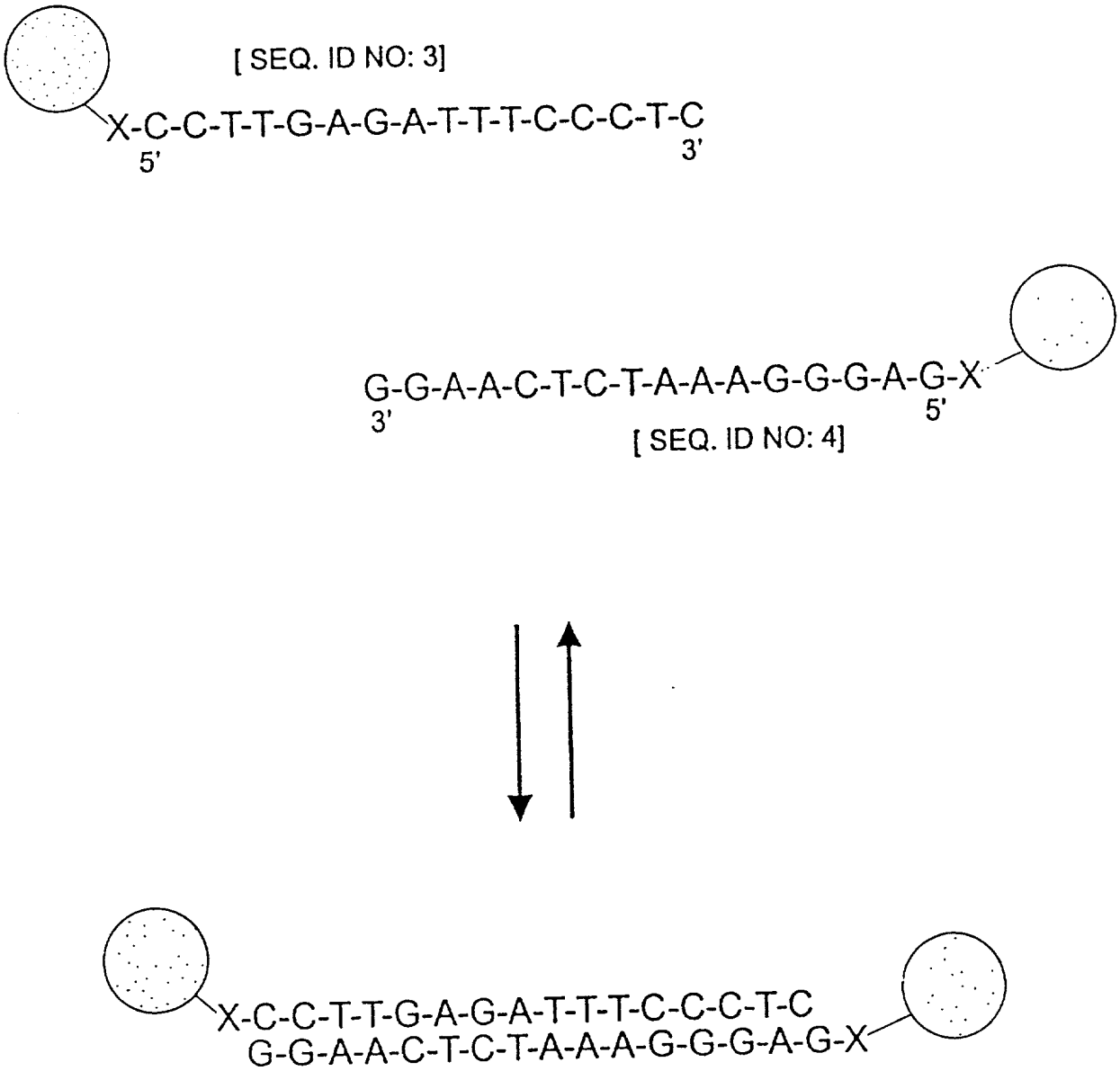

[SEQ. ID NO: 3]
X-C-C-T-T-G-A-G-A-T-T-T-C-C-C-T-C
5'
3'
G-G-A-A-C-T-C-T-A-A-A-G-G-G-A-G-X
3'
5'
[SEQ. ID NO: 4]
X-C-C-T-T-G-A-G-A-T-T-T-C-C-C-T-C
G-G-A-A-C-T-C-T-A-A-A-G-G-G-A-G-X

FIG.2

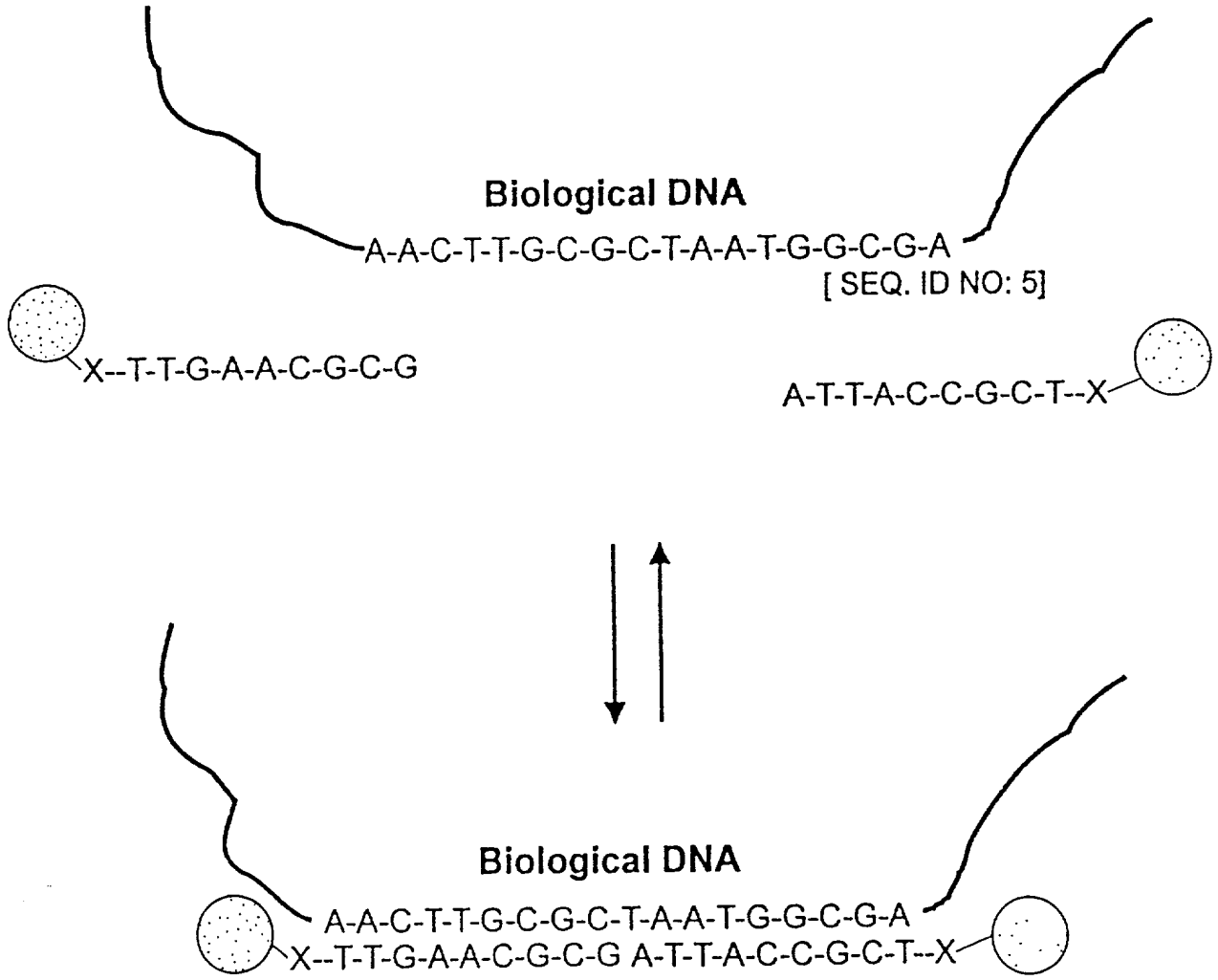

Biological DNA
A-A-C-T-T-G-C-G-C-T-A-A-T-G-G-C-G-A
[SEQ. ID NO: 5]
X--T-T-G-A-A-C-G-C-G
A-T-T-A-C-C-G-C-T--X
Biological DNA
A-A-C-T-T-G-C-G-C-T-A-A-T-G-G-C-G-A
X--T-T-G-A-A-C-G-C-G A-T-T-A-C-C-G-C-T--X

FIG.3

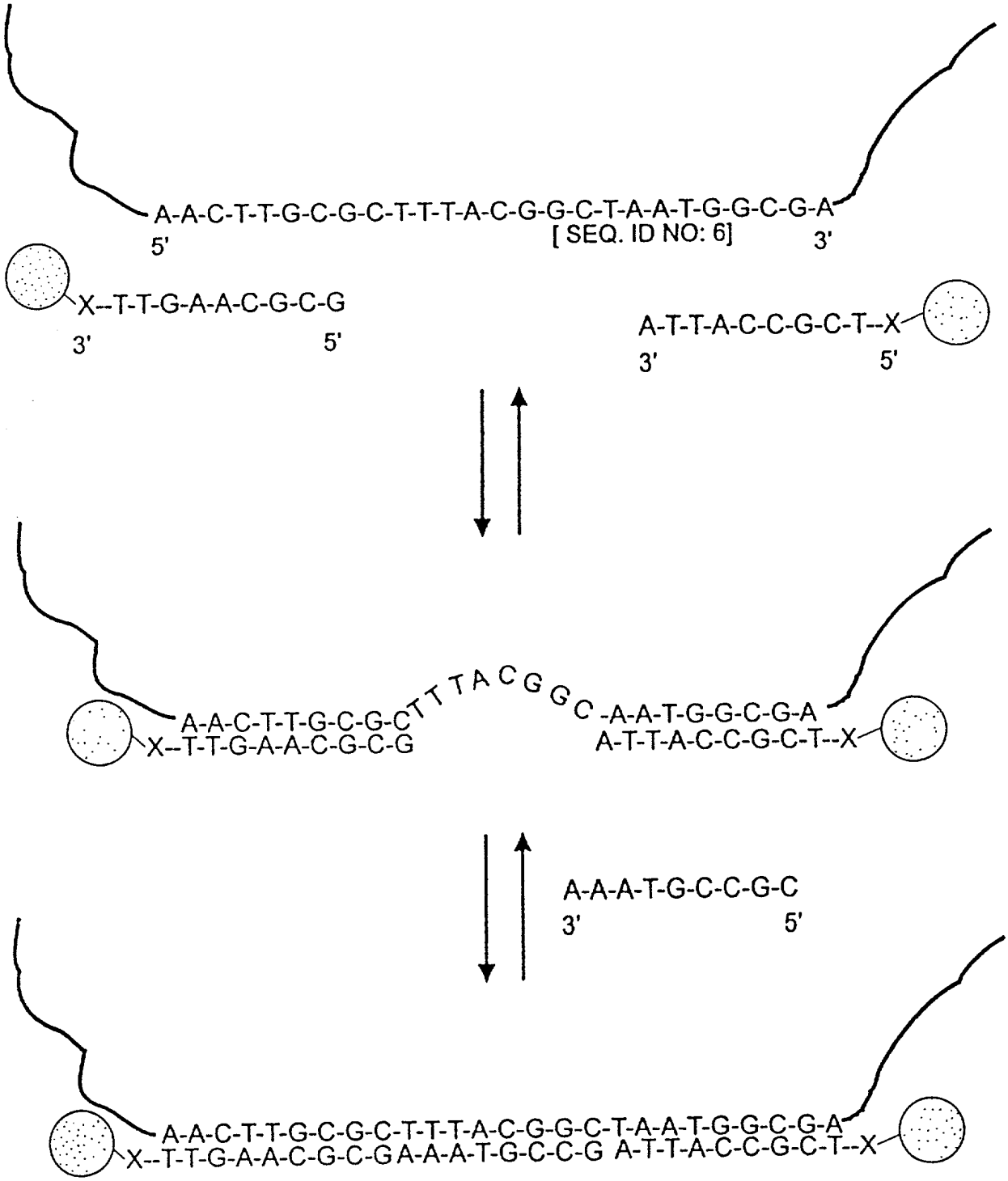

A-A-C-T-T-G-C-G-C-T-T-T-A-C-G-G-C-T-A-A-T-G-G-C-G-A
5'
[SEQ. ID NO: 6]
3'
X--T-T-G-A-A-C-G-C-G
3'
5'
A-T-T-A-C-C-G-C-T--X
3'
5'
A-A-C-T-T-G-C-G-C
X--T-T-G-A-A-C-G-C-G
T T T A C G G C
-A-A-T-G-G-C-G-A
A-T-T-A-C-C-G-C-T--X
A-A-A-T-G-C-C-G-C
3'
5'
A-A-C-T-T-G-C-G-C-T-T-T-A-C-G-G-C-T-A-A-T-G-G-C-G-A
X--T-T-G-A-A-C-G-C-G A-A-A-T-G-C-C-G A-T-T-A-C-C-G-C-T--X

FIG.4

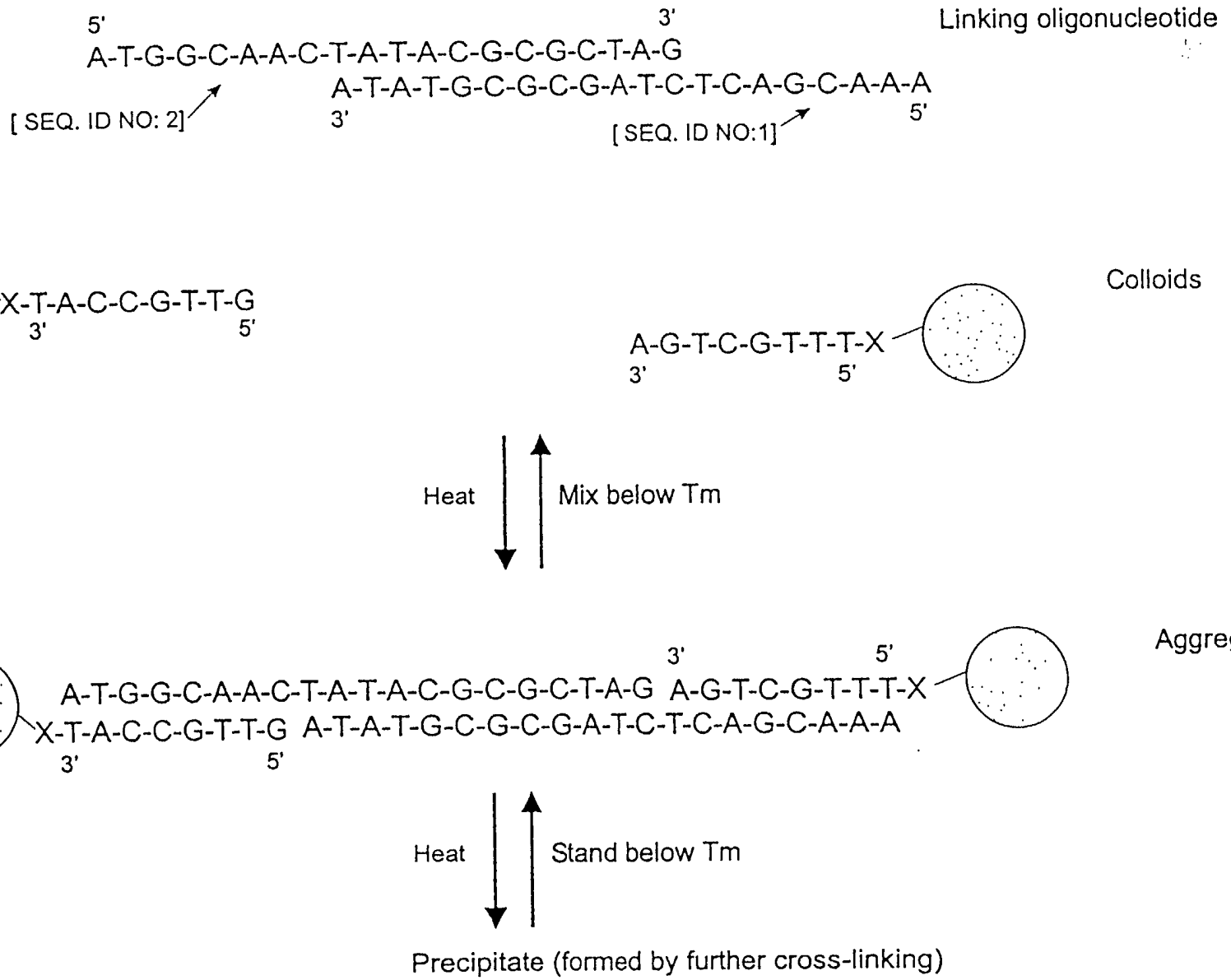

5'
3'
Linking oligonucleotide
A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G
A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A
[SEQ. ID NO: 2]
3'
5'
[SEQ. ID NO:1]
X-T-A-C-C-G-T-T-G
3'
5'
Colloids
A-G-T-C-G-T-T-T-X
3'
5'
Heat
Mix below Tm
Aggregate
3'
5'
A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G A-G-T-C-G-T-T-T-X
X-T-A-C-C-G-T-T-G A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A
3'
5'
Heat
Stand below Tm
Precipitate (formed by further cross-linking)

FIG.5

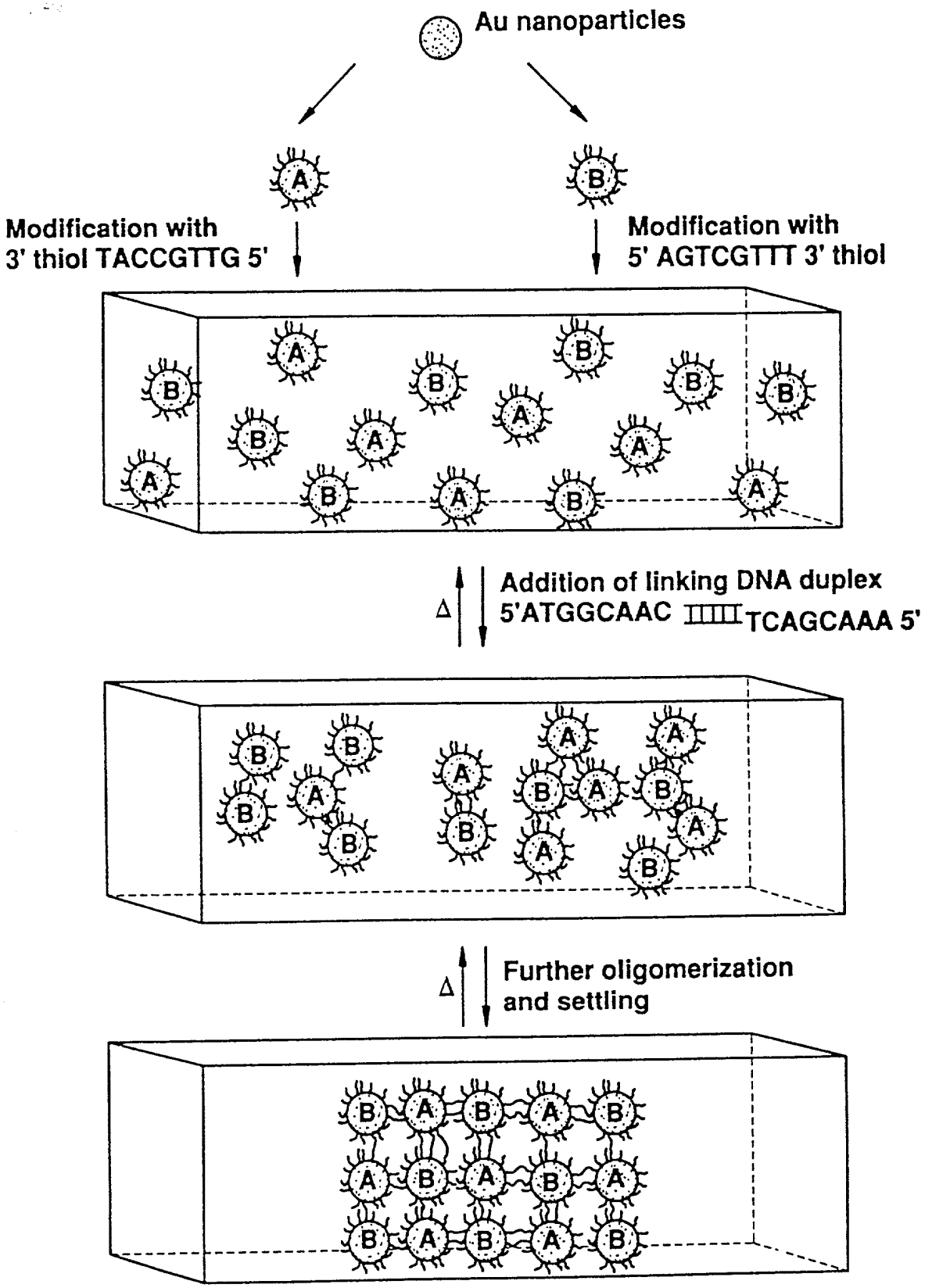

Au nanoparticles
A
B
Modification with
3' thiol TACCGTTG 5'
Modification with
5' AGTCGTTT 3' thiol
Δ
Addition of linking DNA duplex
5'ATGGCAAC TCAGCAAA 5'
Δ
Further oligomerization
and settling

FIG.6A FIG.6B FIG.6C

FIG. 7

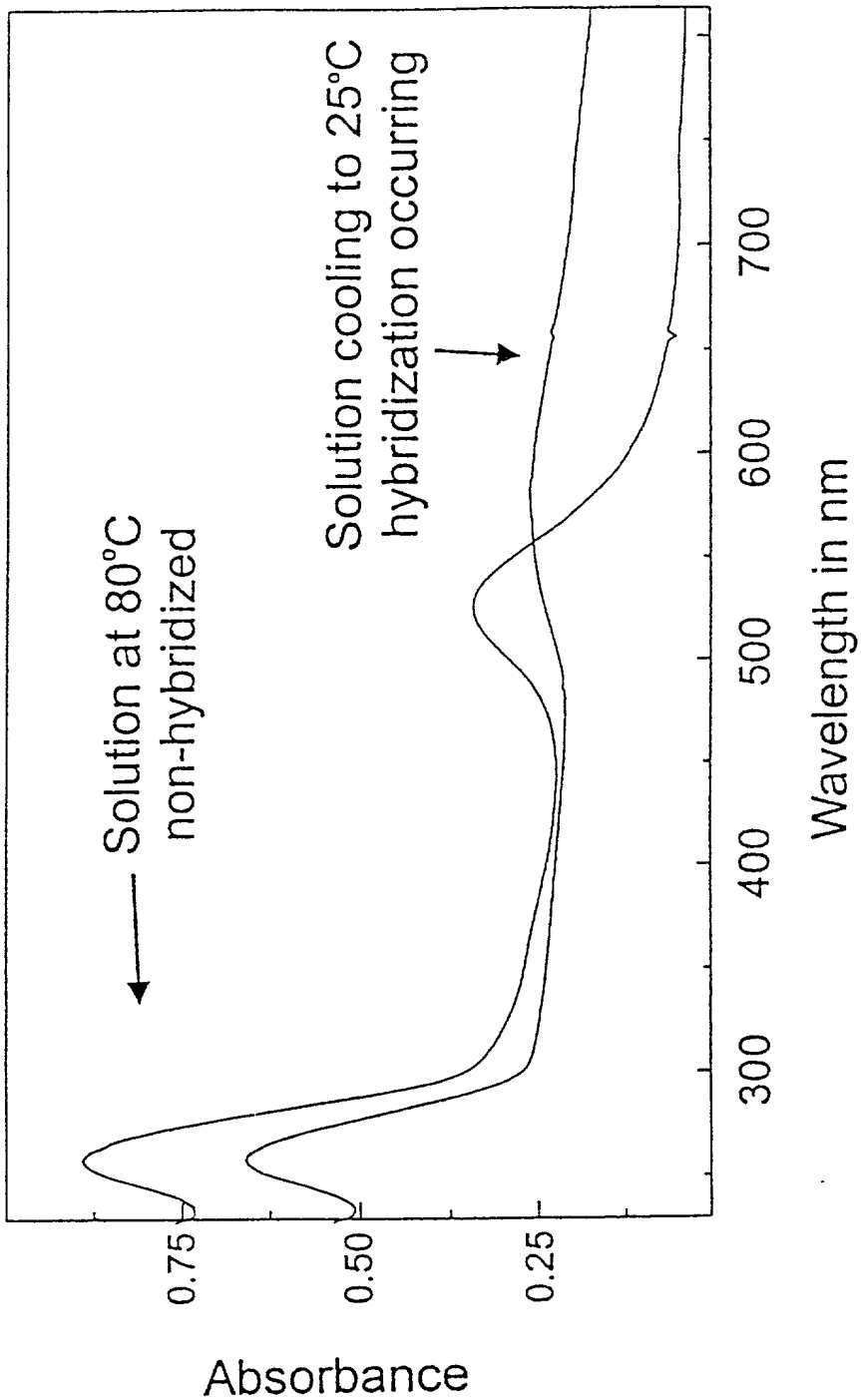

Solution at 80°C
non-hybridized
Solution cooling to 25°C
hybridization occurring
0.75
0.50
0.25
Absorbance
300
400
500
600
700
Wavelength in nm

FIG.8A

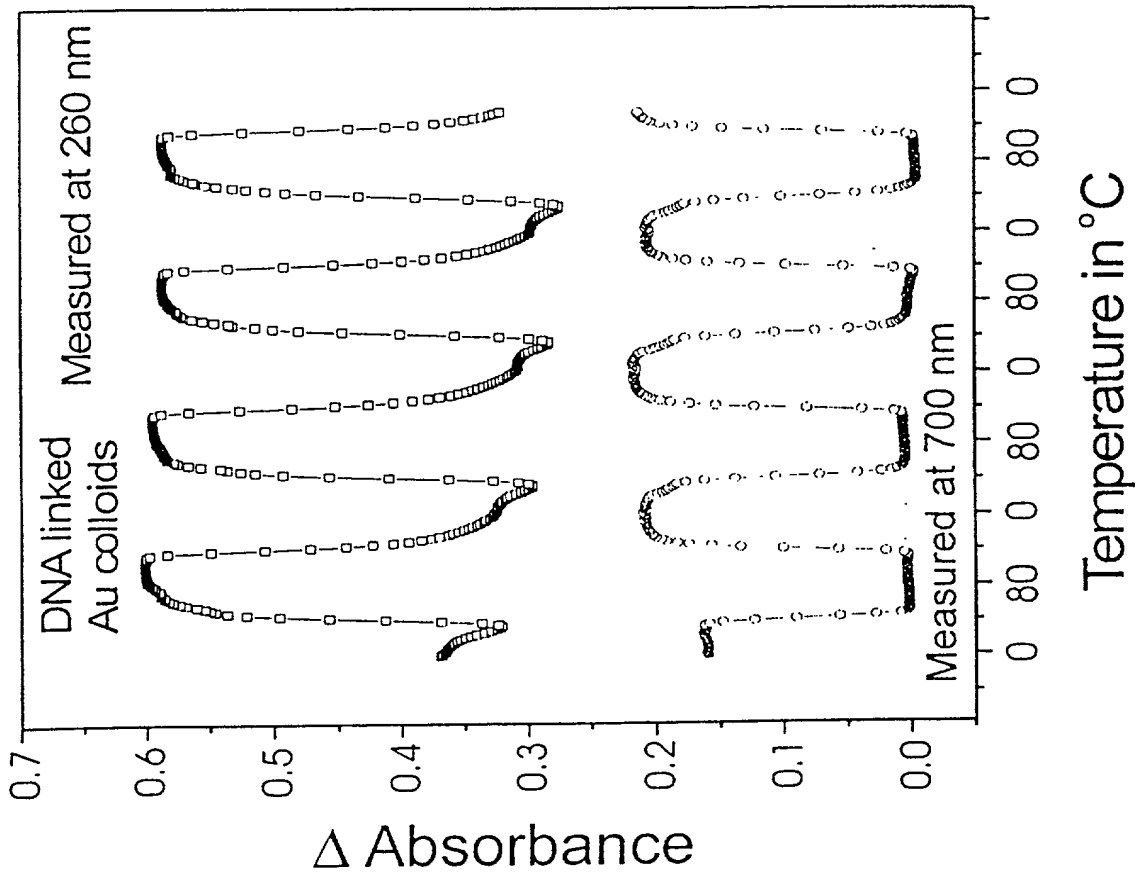

DNA linked
Au colloids
Measured at 260 nm
Measured at 700 nm
Δ Absorbance
Temperature in °C
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 80 0 80 0 80 0 80 0 80 0

FIG.8B

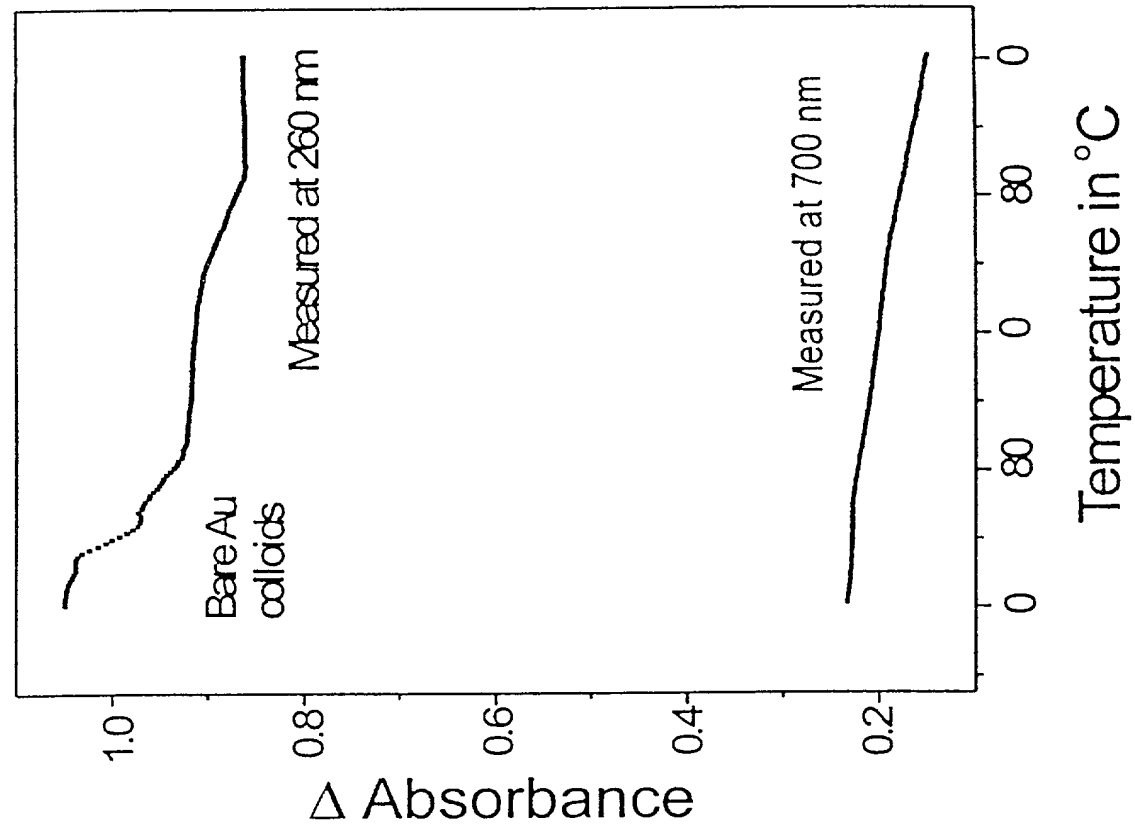

Bare Au
colloids
Measured at 260 nm
Measured at 700 nm
Δ Absorbance
Temperature in °C
1.0
0.8
0.6
0.4
0.2
0 80 0 80 0 80 0

FIG.9A

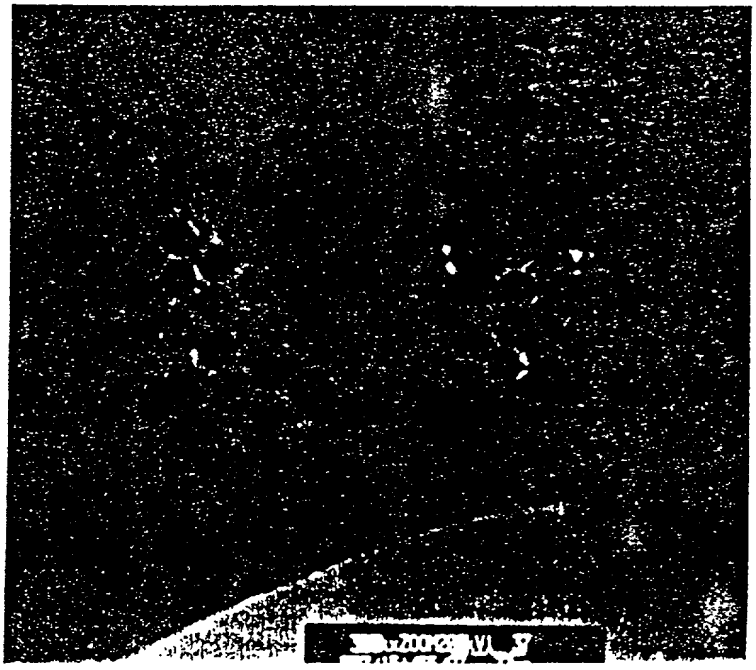


FIG.9B

FIG.10

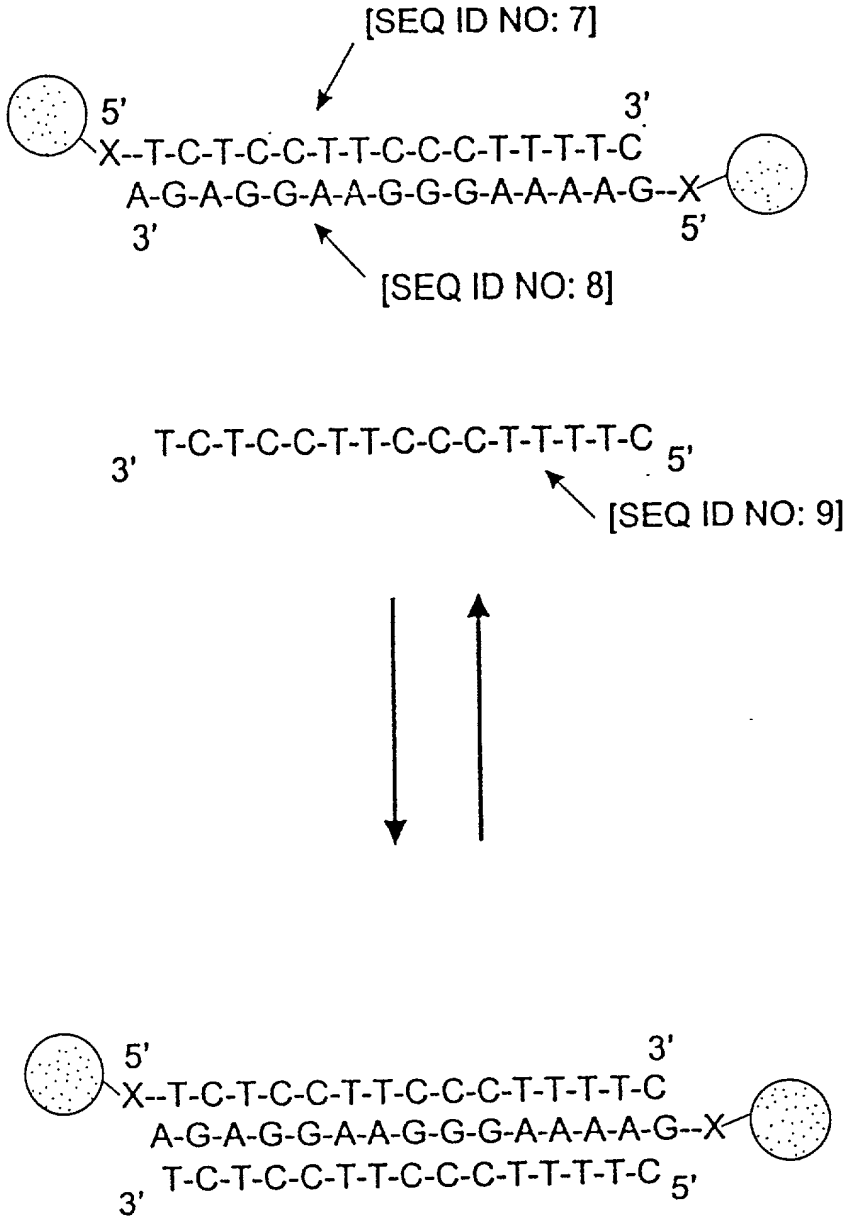

[SEQ ID NO: 7]
5'
3'
X--T-C-T-C-C-T-T-C-C-C-T-T-T-T-C
A-G-A-G-G-A-A-G-G-G-A-A-A-A-G--X
3'
5'
[SEQ ID NO: 8]
3'
T-C-T-C-C-T-T-C-C-C-T-T-T-T-C
5'
[SEQ ID NO: 9]
5'
3'
X--T-C-T-C-C-T-T-C-C-C-T-T-T-T-C
A-G-A-G-G-A-A-G-G-G-A-A-A-A-G--X
3'
T-C-T-C-C-T-T-C-C-C-T-T-T-T-C
5'

FIG. 11

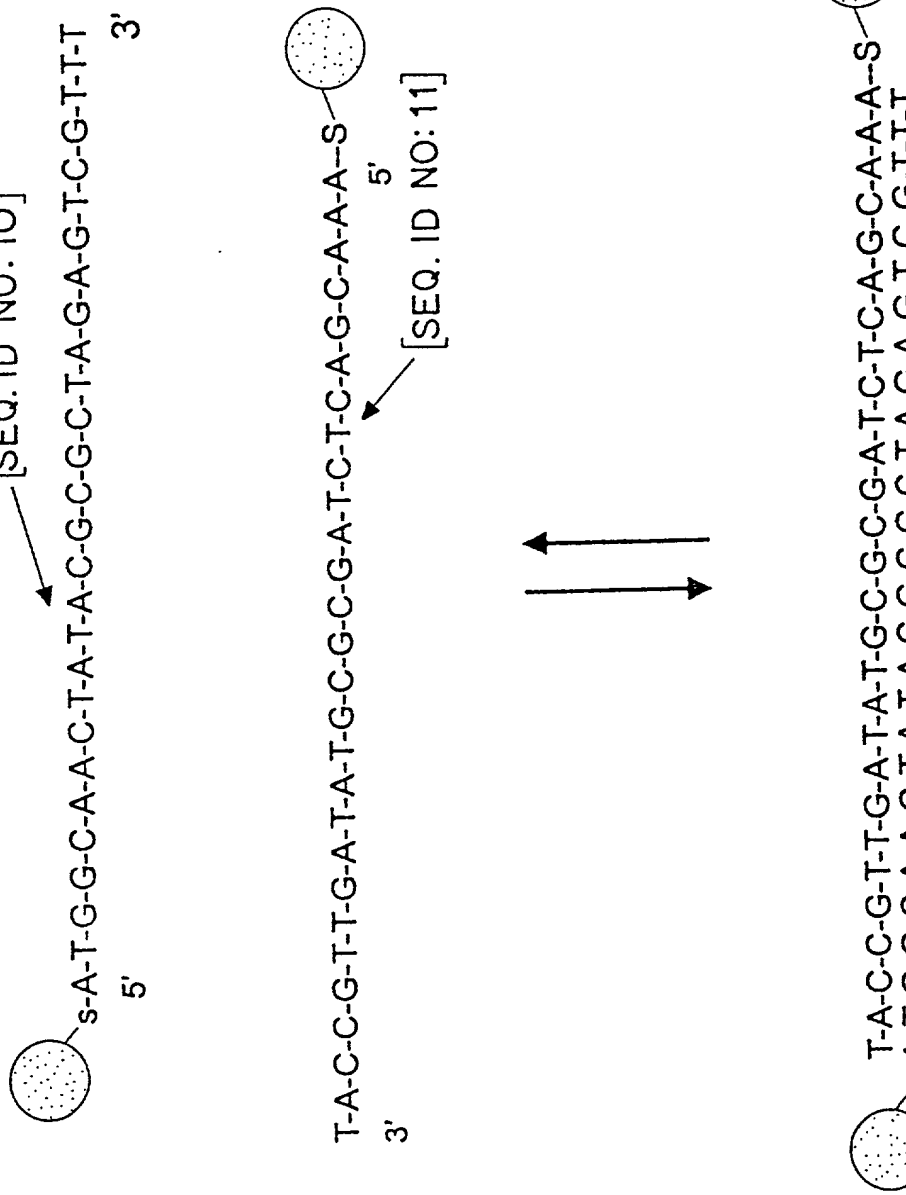

[SEQ. ID NO: 10]
s-A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G-A-G-T-C-G-T-T-T
5'
3'
T-A-C-C-G-T-T-G-A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A-S
3'
5'
[SEQ. ID NO: 11]
T-A-C-C-G-T-T-G-A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A-S
s-A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G-A-G-T-C-G-T-T-T

FIG.12A

Complementary Target

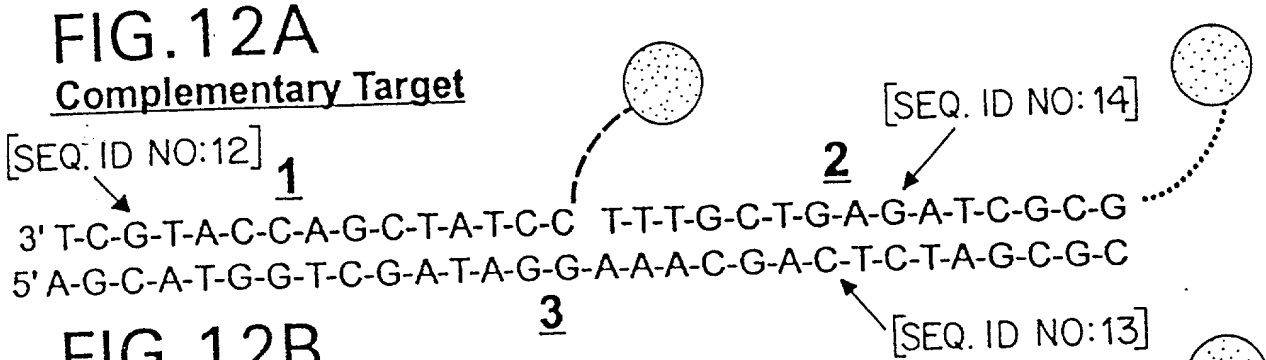


FIG.12B

Probes without Target

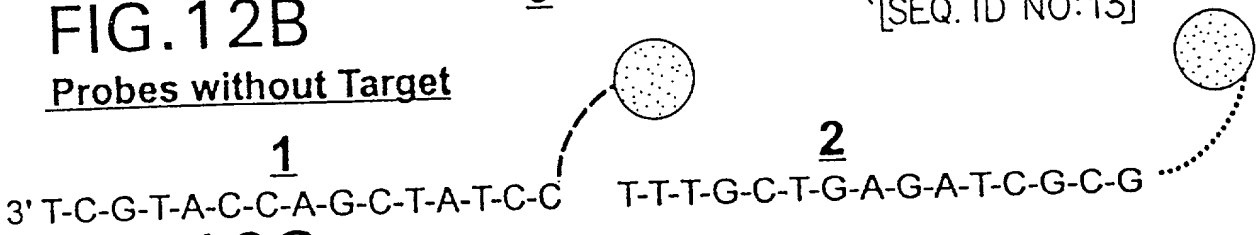


FIG.12C

Half Complementary Target

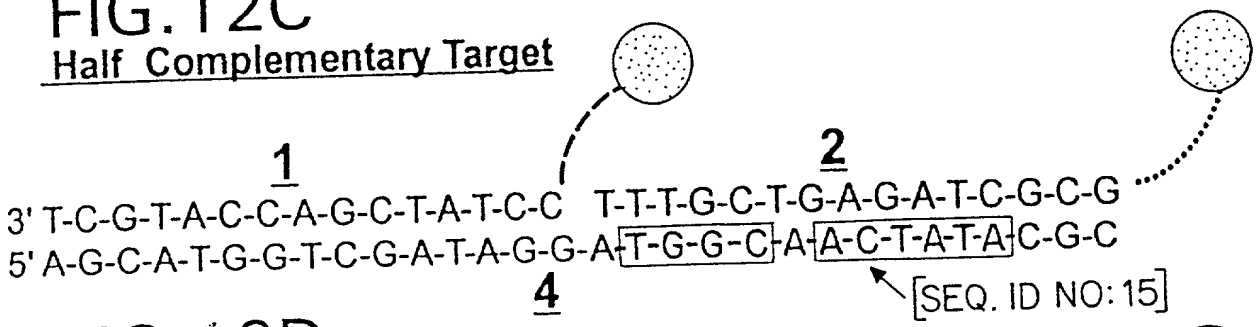


FIG.12D

Target - 6 bp

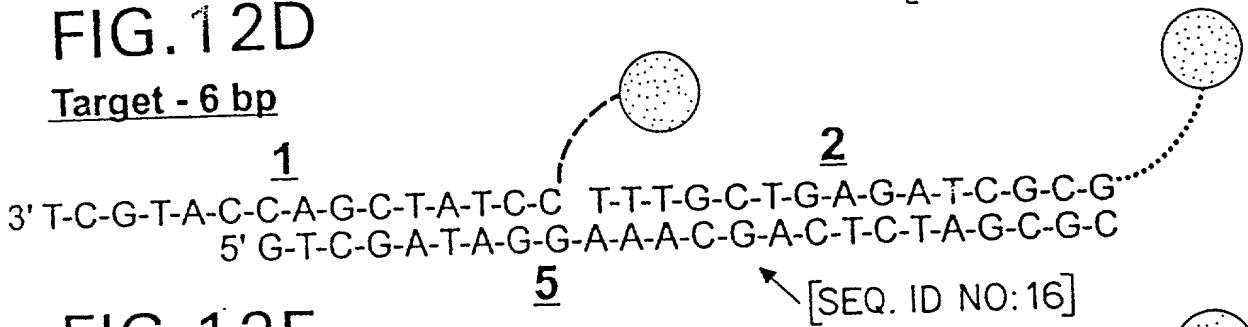


FIG.12E

One bp Mismatch

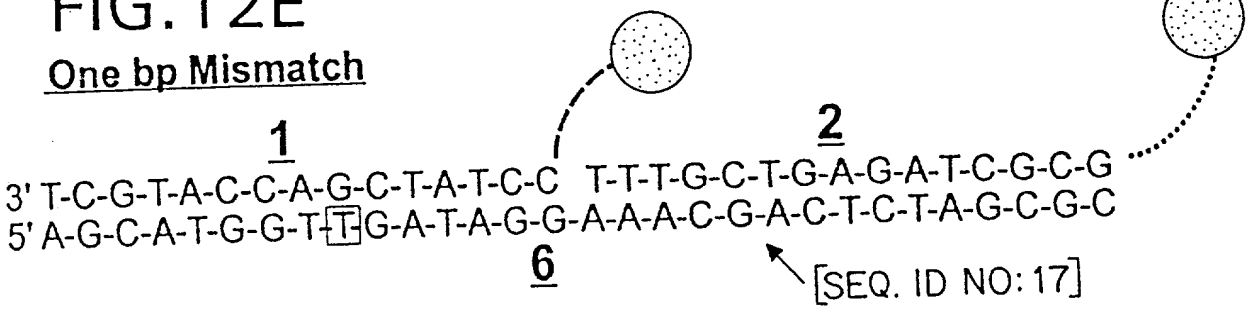


FIG.12F

Two bp Mismatch

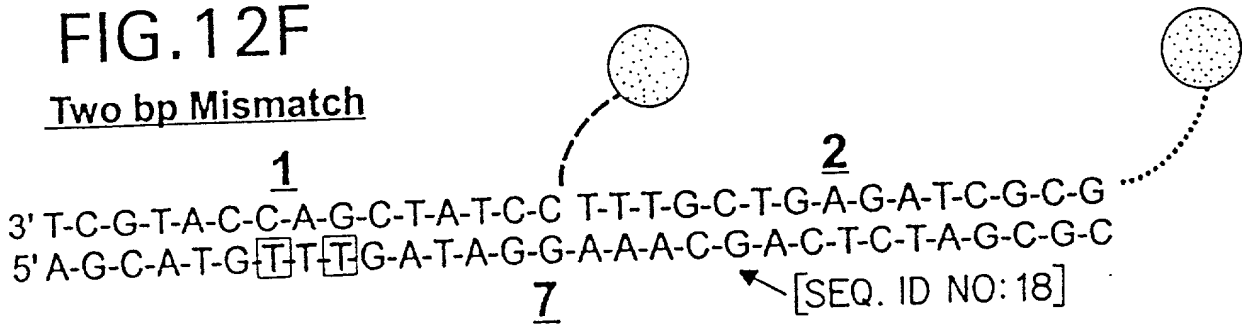
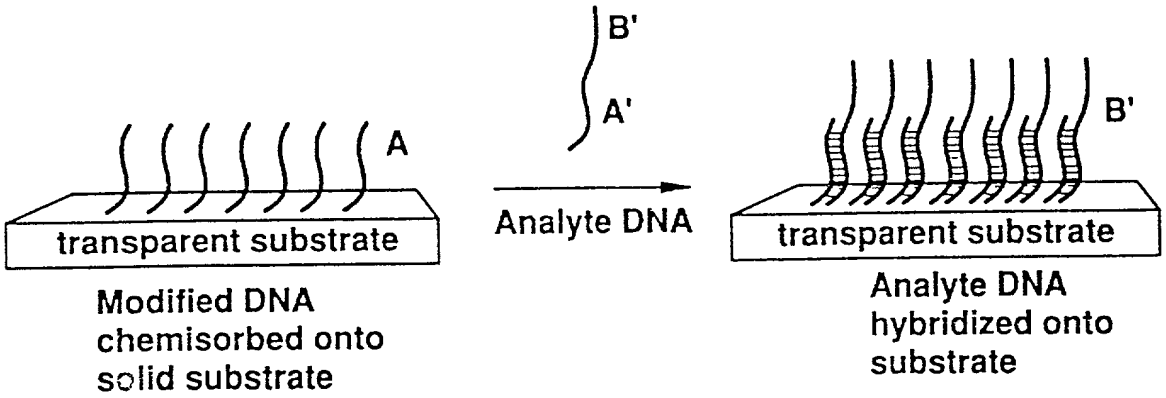
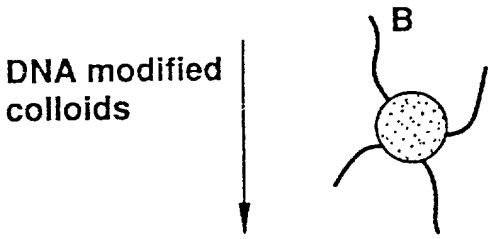


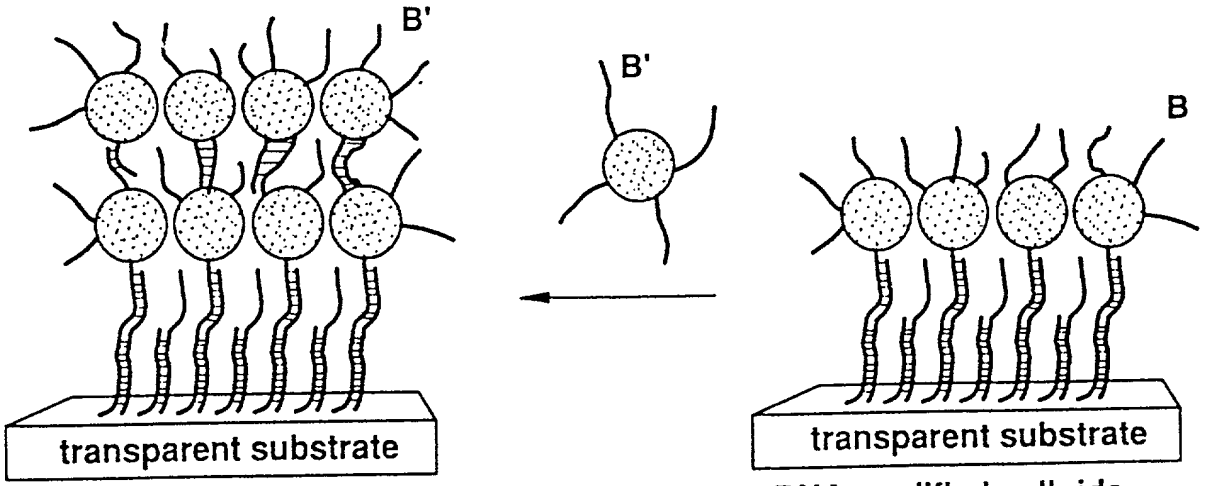
FIG.13A



Modified DNA chemisorbed onto solid substrate

Analyte DNA hybridized onto substrate





Dark areas where nanoparticle aggregates are linked to substrate surface by analyte DNA

DNA modified colloids hybridized to bound analyte DNA

FIG.13B

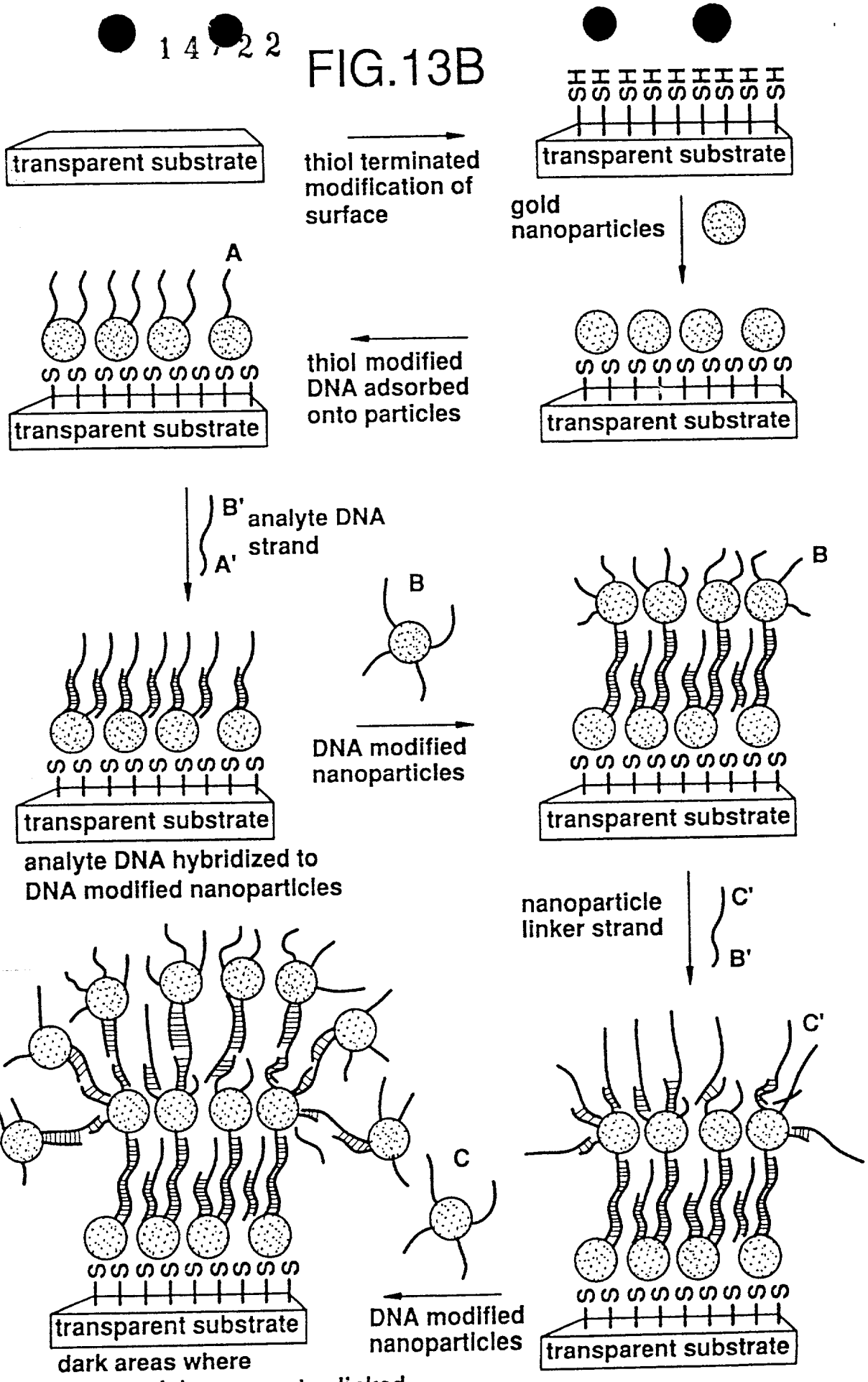

transparent substrate
thiol terminated modification of surface
transparent substrate
gold nanoparticles
transparent substrate
thiol modified DNA adsorbed onto particles
A
transparent substrate
B'
A'
analyte DNA strand
B
DNA modified nanoparticles
transparent substrate
analyte DNA hybridized to DNA modified nanoparticles
B
transparent substrate
nanoparticle linker strand
C'
B'
C'
C
transparent substrate
DNA modified nanoparticles
transparent substrate
dark areas where nanoparticle aggregates linked to substrate by analyte DNA

FIG.14A

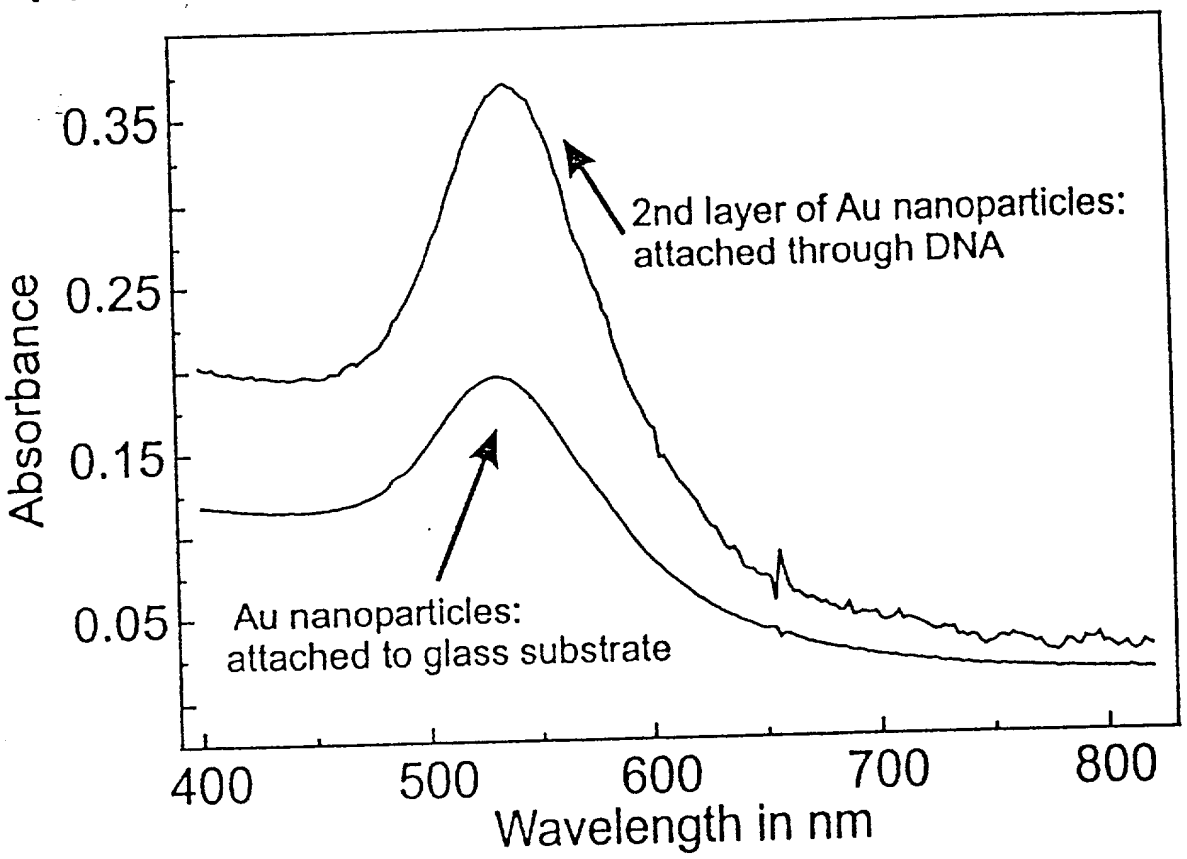


FIG.14B

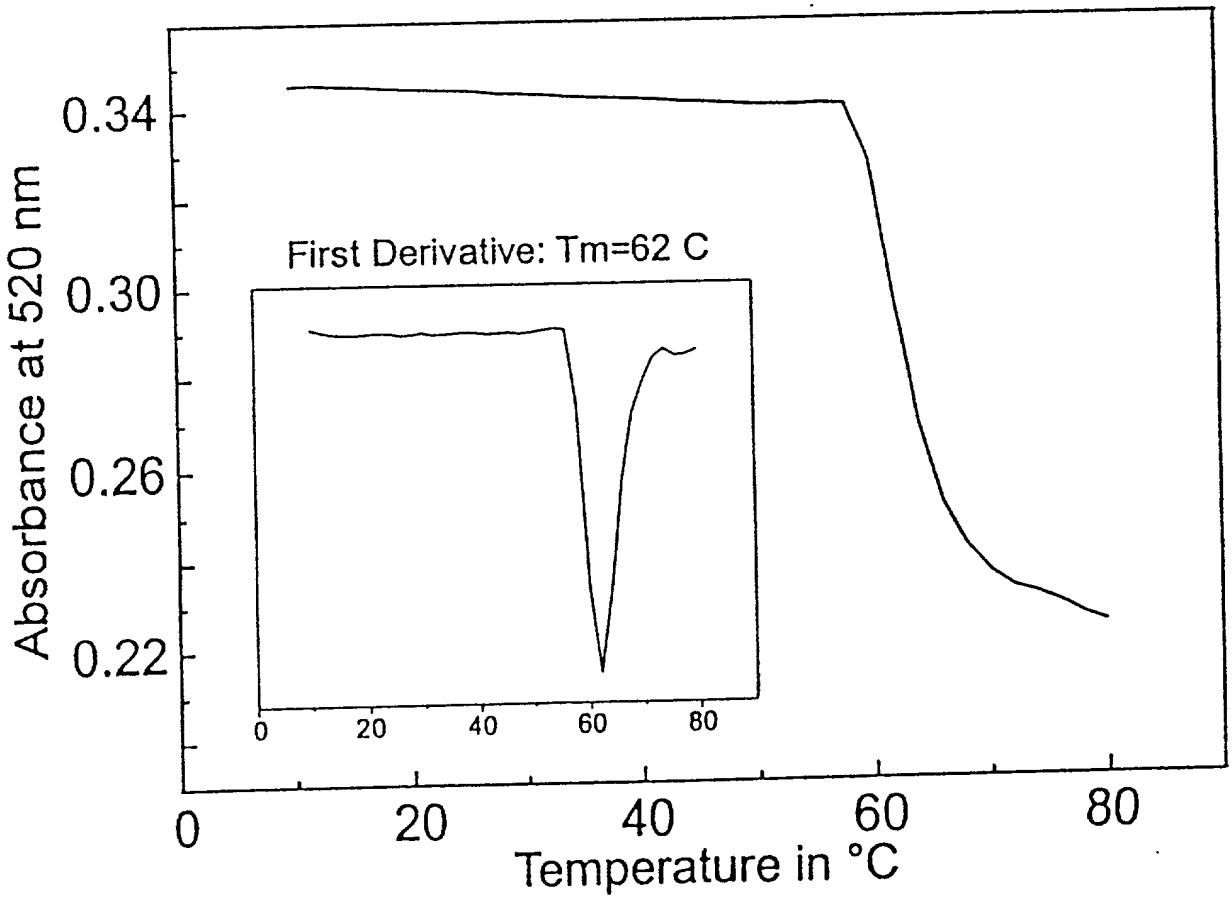


FIG15A

Probes with No Target

SEQ ID NO:19

SEQ ID NO:20

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1 2

FIG15B

Half-Complementary Target

3

SEQ ID NO:21

5' TAC-GAG-TTG-AGA-GAG-TGC-CCA-CAT 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1 2

FIG15C

Complementary Target

Tm=53.5°C

4

SEQ ID NO:22

5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1 2

FIG15D

ONE Base-Pair Mismatch at Probe Head

Tm=50.4°C

5

SEQ ID NO:23

5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCT 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1 2

FIG15E

Tm=46.2°C

ONE Base-Pair Mismatch at Probe Tail

6

SEQ ID NO:24

5' TAC-GAG-TTG-AGA-CTC-CTG-AAT-GCG 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1 2

FIG15F

Tm=51.6°C

ONE Base Deletion

7

SEQ ID NO:25

5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GC□ 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1 2

FIG15G

Tm=50.2°C

ONE Base-Pair Insertion

8

SEQ ID NO:26

5' TAC-GAG-TTG-AGA-CAT-CCT-GAA-TGC-G 3'

S-ATG-CTC-AAC-TCT TA-GGA-CTT-ACG-C-S

1 2

FIG.16A **24 Base Template**

5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3'
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S
1 2

FIG.16B **48 Base Template with Complementary 24 Base Filler**

5' TAC-GAG-TTG-AGA-CCG-TTA-AGA-CGA-GGC-AAT-CAT-GCA-ATC-CTG-AAT-GCG 3'
S-ATG-CTC-AAC-TCT GGC-AAT-TCT-GCT-CCG-TTA-GTA-CGT TAG-GAC-TTA-CGC-S
1 2

FIG.16C **72 Base Template with Complementary 48 Base Filler**

5' TAC-GAG-TTG-AGA-CCG-TTA-AGA-CGA-GGC-AAT-CAT-GCA-TAT-ATT-GGA-CGC-TTT-ACG-GAC-AAC-ATC-CTG-AAT-GCG 3'
S-ATG-CTC-AAC-TCT GGC-AAT-TCT-GCT-CCG-TTA-GTA-CGT-ATA-TAA-CCT-GCG-AAA-TGC-CTG-TTG TAG-GAC-TTA-CGC-S
1 2

FIG.17A

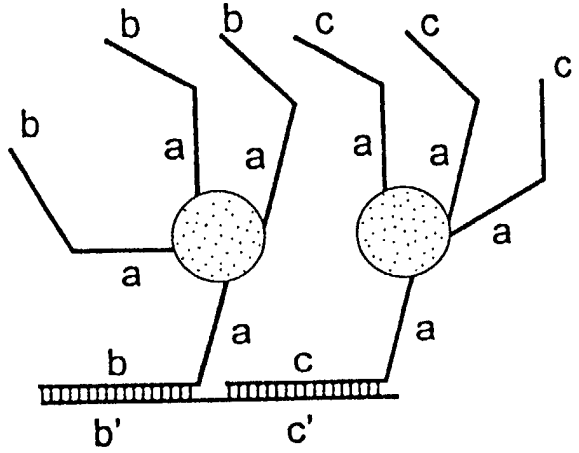


FIG.17B

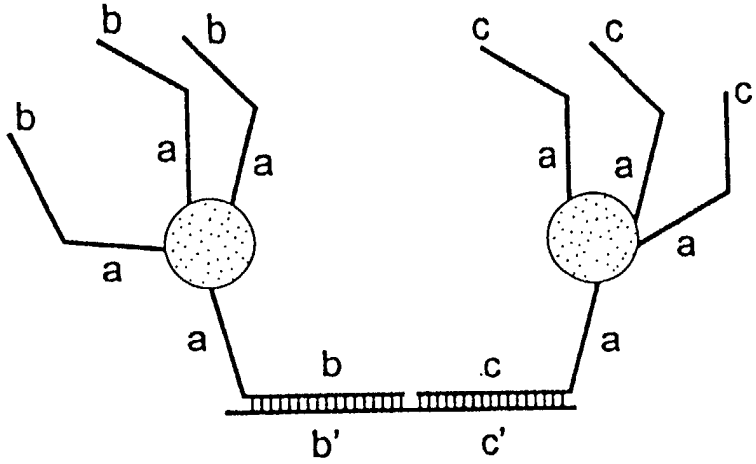


FIG.17C

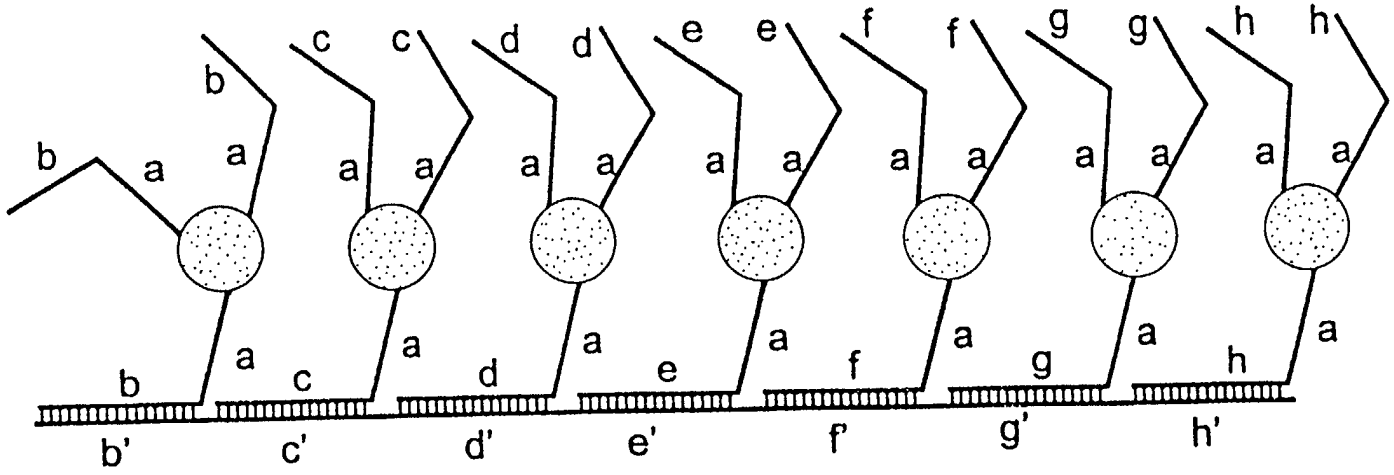


FIG.17D

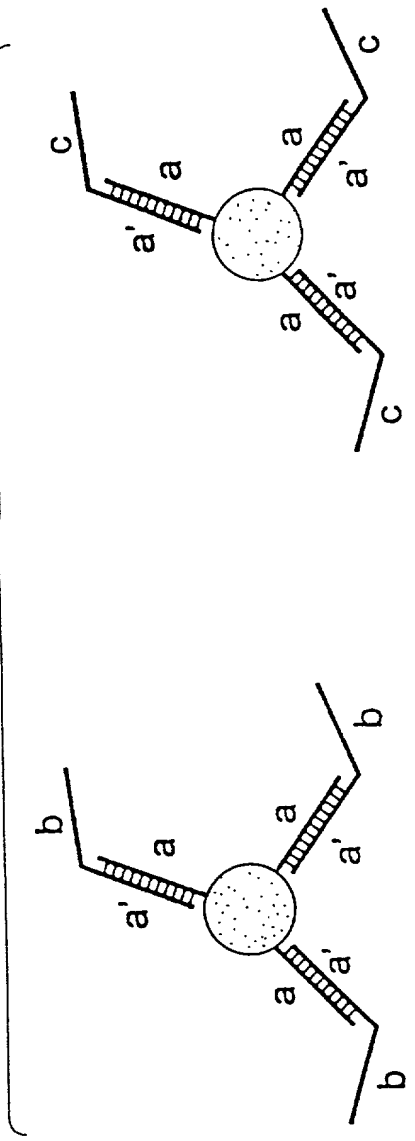


FIG.17E

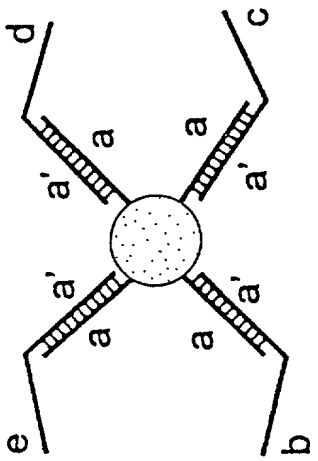


FIG.18

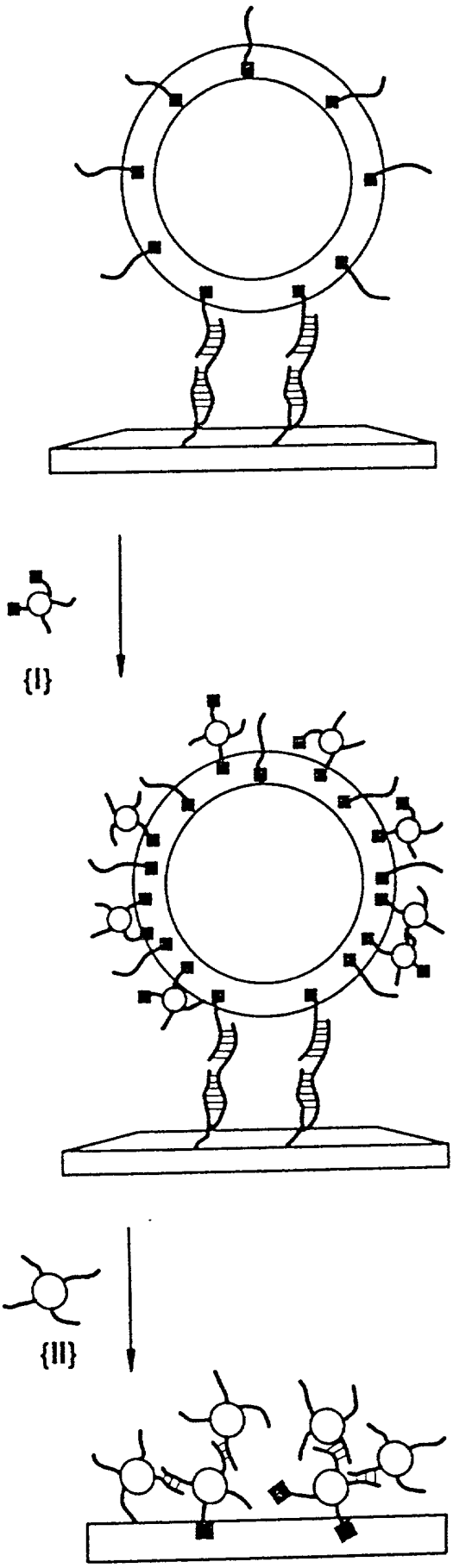


FIG. 19A

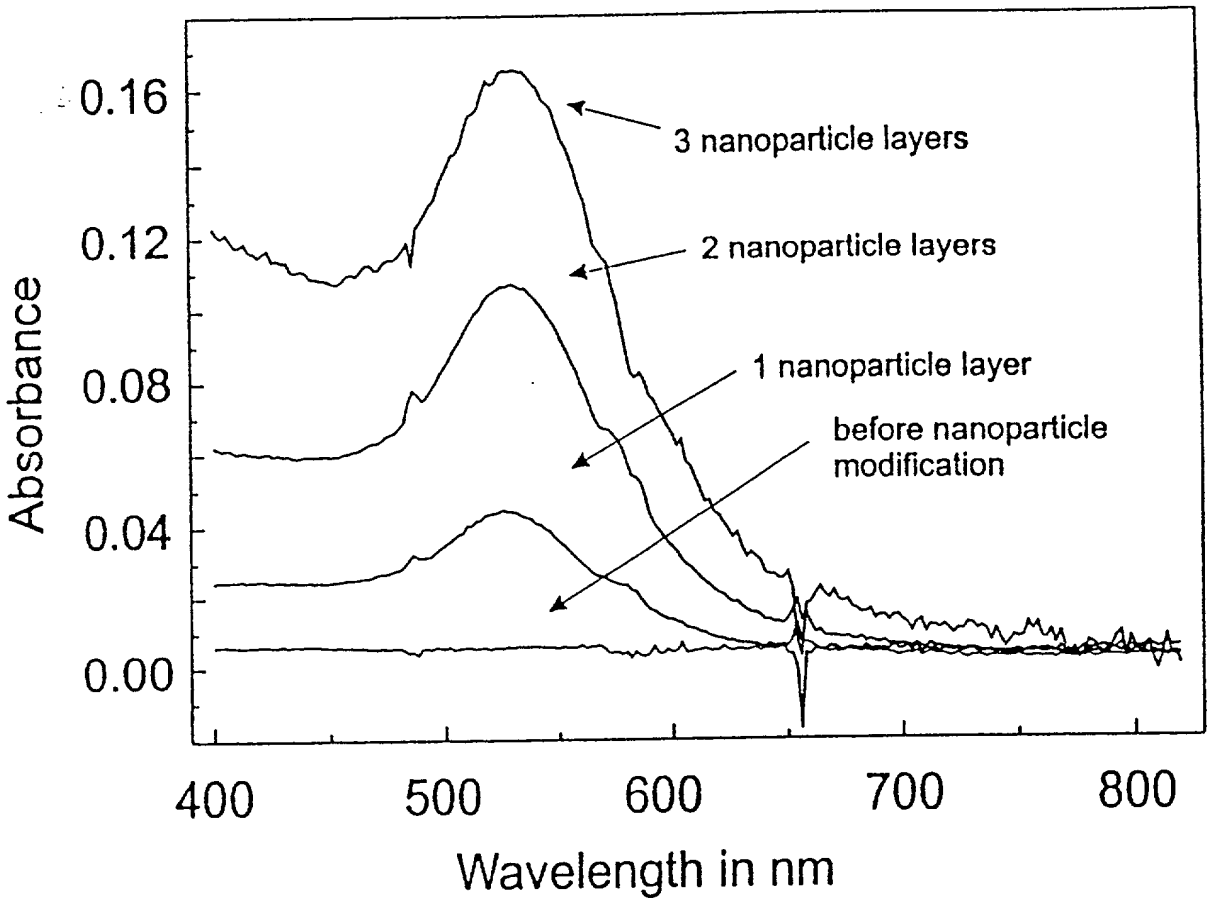


FIG. 19B

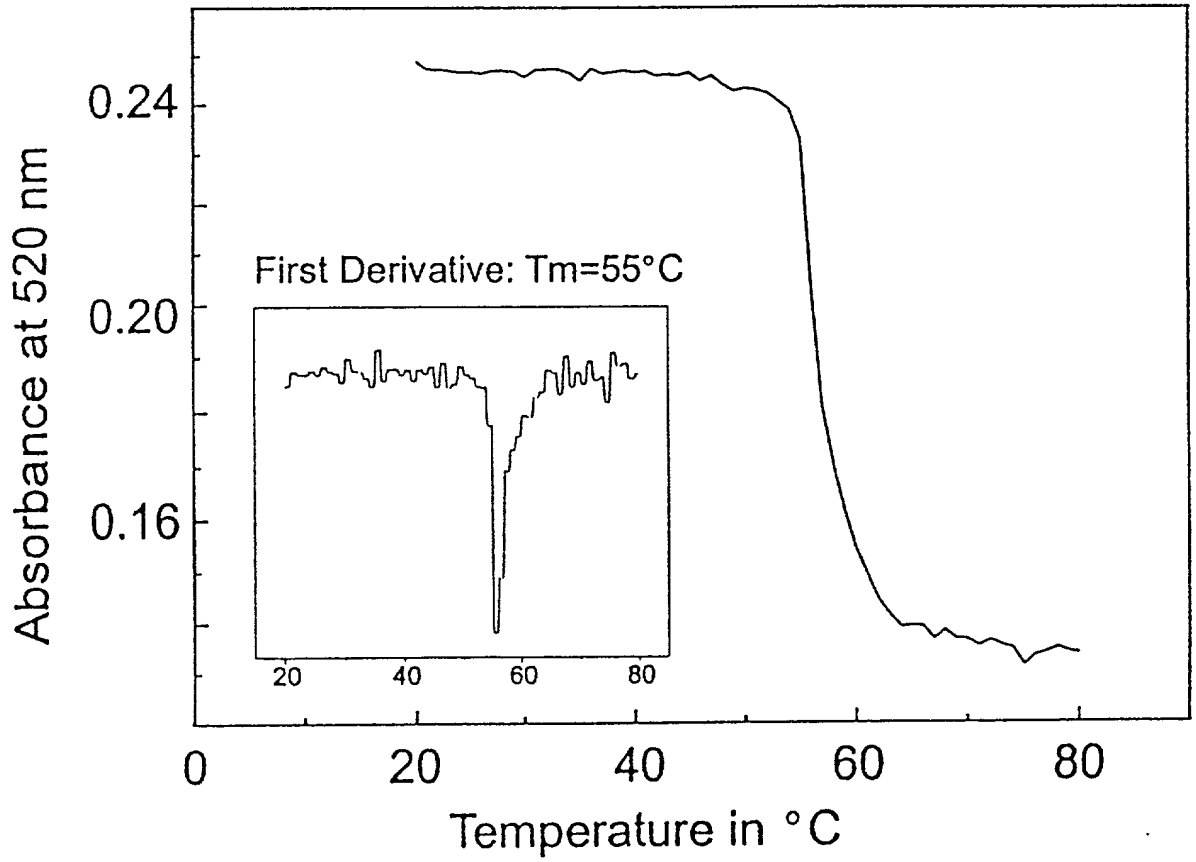


FIG.20A

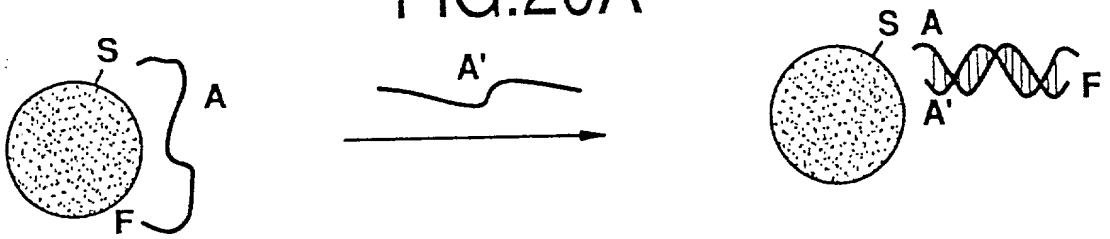
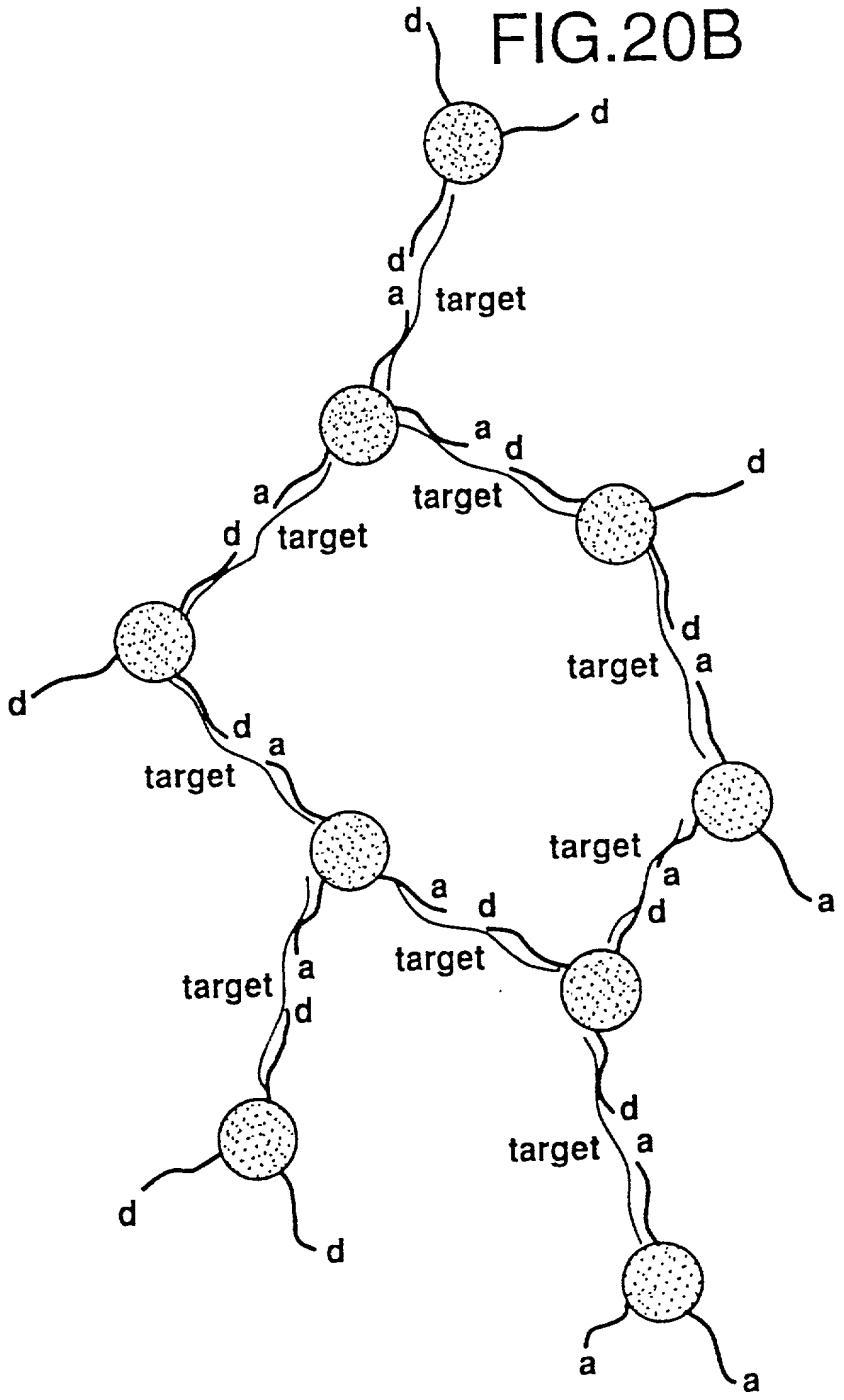


FIG.20B



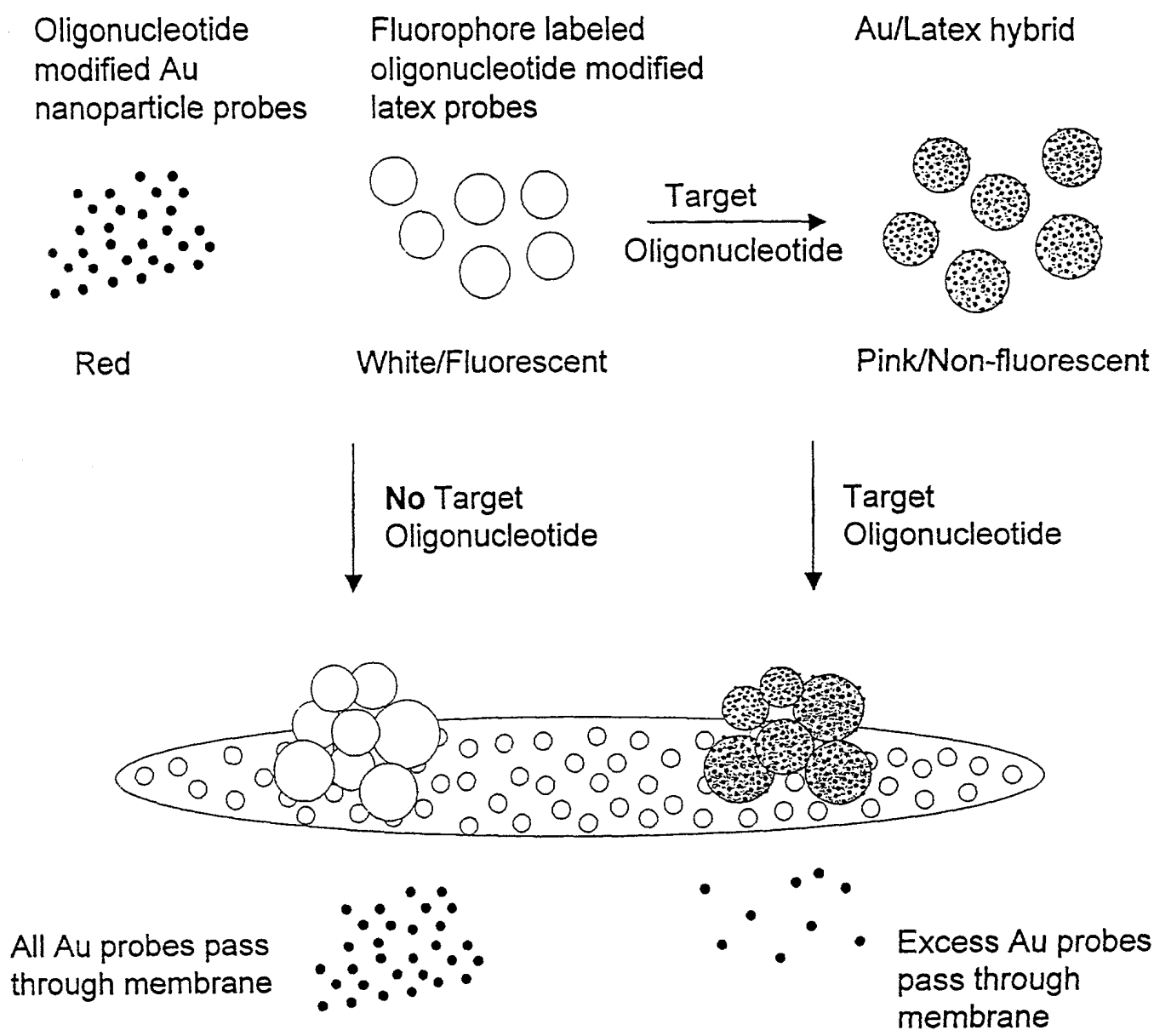
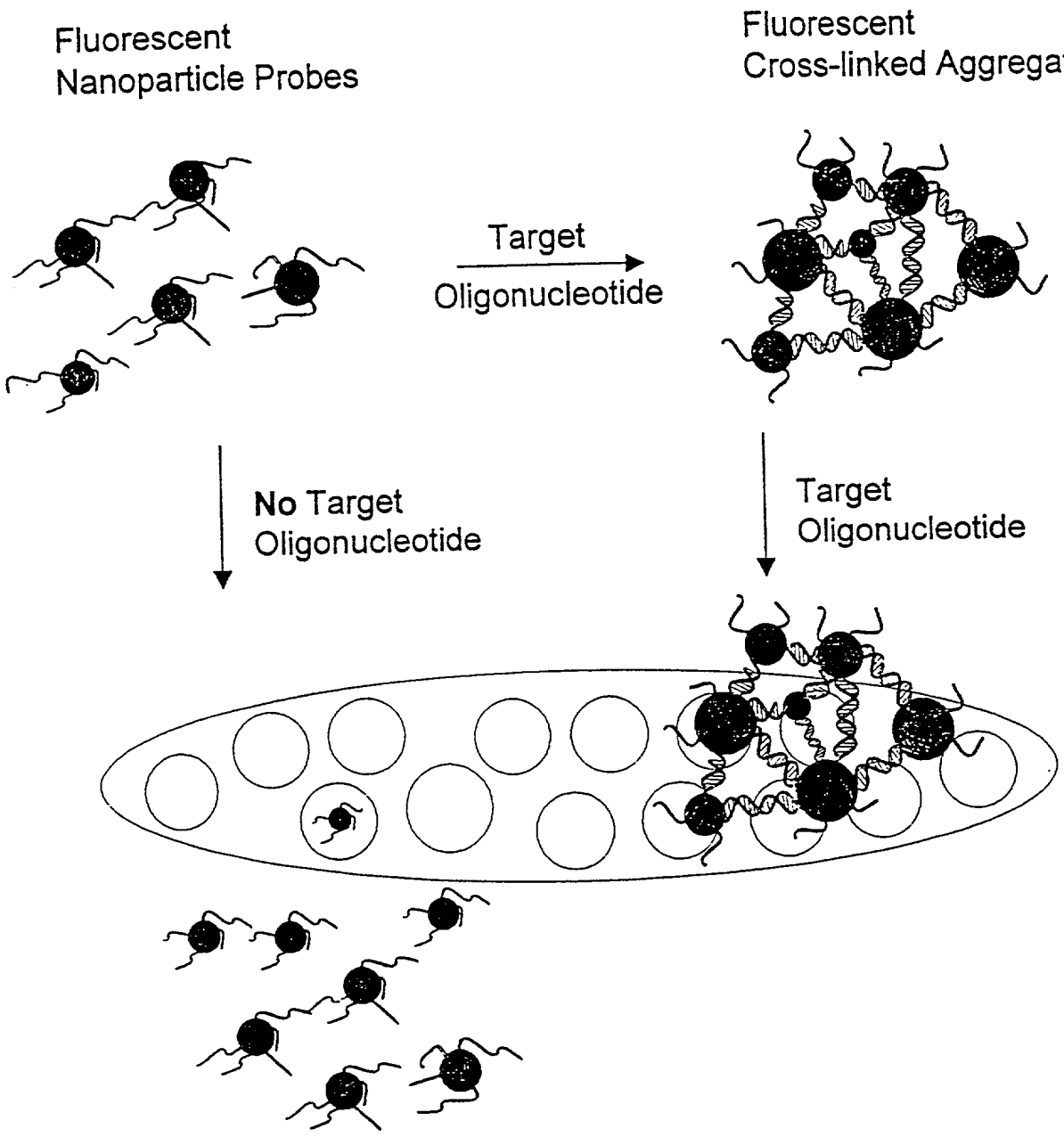

Oligonucleotide modified Au nanoparticle probes
Fluorophore labeled oligonucleotide modified latex probes
Au/Latex hybrid
Target Oligonucleotide
Red
White/Fluorescent
Pink/Non-fluorescent
No Target Oligonucleotide
Target Oligonucleotide
All Au probes pass through membrane
Excess Au probes pass through membrane

FIGURE 21

FIGURE 22



The fluorescent nanoparticle probes pass through the membrane

The fluorescent cross-linked aggregates are retained by the membrane

Anthrax PCR Product

5'G GCG GAT GAG TCA GTA GTT AAG GAG GCT CAT AGA GAA GTA ATT AAT
3'C CGC CTA CTC AGT CAT CAA TTC CTC CGA GTA TCT CTT CAT TAA TTA

TCG TCA ACA **GAG GGA TTA TTG TTA AAT ATT GAT AAG GAT** ATA AGA AAA
AGC AGT TGT CTC CCT AAT AAC AAT TTA TAA CTA TTC CTA TAT TCT TTT

ATA TTA TCC AGG GTT ATA TTG TAG AAA TTG AAG ATA CTG AAG GGC TT 3'
TAT AAT AGG TCC CAA TAT AAC ATC TTT AAC TTC TAT GAC TTC CCG AA 5'

141 mer Anthrax PCR product [SEQ ID NO:36]

3' CTC CCT AAT AAC AAT
[SEQ ID NO:37]

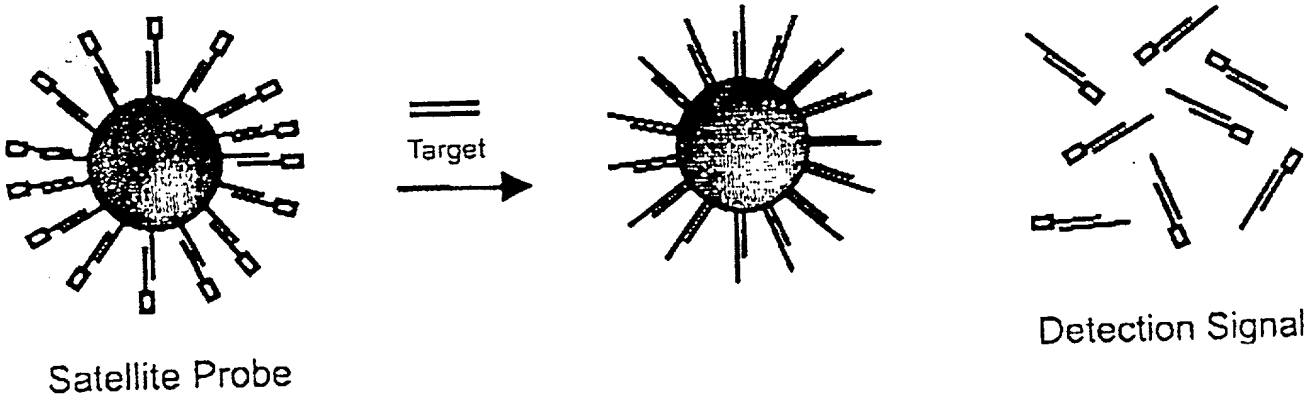
3' TTA TAA CTA TTC CTA
[SEQ ID NO:38]

Oligonucleotide-Nanoparticle Probes

Blocker Oligonucleotides

3' C CGC CTA CTC AGT CAT CAA TTC CTC CGA GT	[SEQ ID NO:39]
3' A TCT CTT CAT TAA TTA AGC AGT TGT	[SEQ ID NO:40]
3' TAT TCT TTT TAT AAT AGG TCC CAA TAT	[SEQ ID NO:41]
3' AAC ATC TTT AAC TTC TAT GAC TTC CCG AA	[SEQ ID NO:42]

FIGURE 23


Target
Satellite Probe
Detection Signal

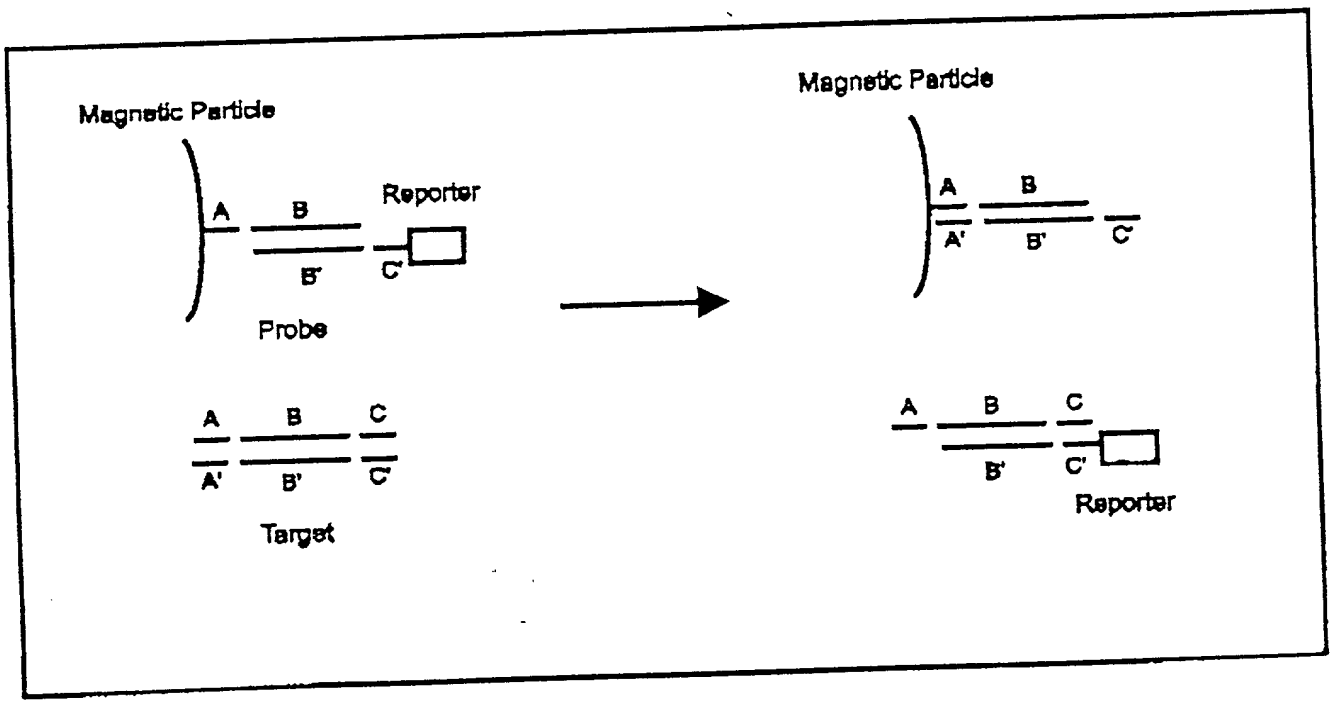

Magnetic Particle
A
B
Reporter
B'
C'
Probe
A
B
C
A'
B'
C'
Target
Magnetic Particle
A
B
A'
B'
C'
A
B
C
B'
C'
Reporter

FIGURE 24